S7.P20

Role of mitochondria in reactive oxygen species production and inflammatory processes in endothelial cells

Antoni Wrzosek[a], Agata Skup[a], Agnieszka Lukaisak[b], Adam Szewczyk[a]
[a]*Nencki Institute of Experimental Biology, Department of Biochemistry, Laboratory of Intracellular Ion Channels, Poland*
[b]*Warsaw University of Life Science, SGGW, Department of Biophysics, 159 Nowoursynowska Street, Poland*
E-mail address: antoni@nencki.gov.pl

Energy requirements in endothelial cells are relatively low, and glycolysis is the major source of ATP production. For these reasons, the mitochondria of endothelial cells have been somewhat neglected. Recently it was discovered that mitochondria, and especially mitochondria in endothelial cells, predominantly function in signalling cellular responses to environmental signals. Important modes of mitochondrial signalling in endothelial cells are the regulated production of reactive oxygen species (ROS) and signalling, autophagy, calcium homeostasis, apoptosis, and heme biosynthesis. It is now clear that mitochondria are important in endothelial physiology and pathophysiology. It is also well documented that inflammation is a key factor that accelerates the onset and progression of atherosclerosis. In our study we have investigated the role of endothelial cell mitochondria in regulation of ROS and NO production, and inflammatory response. As a model of mitochondrial dysfunction endothelial cells EA.hy 926 modified with 2′,3′-Dideoxycytidine (ddc) were used. Inflammation was stimulated with tumour necrosis factor alpha (TNF-alpha) in dose and time dependent manner. The ROS and NO production were measured using fluorescence dyes methods. Intercellular adhesion molecule 1 (ICAM-1) expression in normal and modified endothelial cells were detected using confocal microscopy, flow cytometry, fluorescence techniques at protein level and real time PCR at mRNA level. We demonstrated that EA.hy 926 cells treated with ddc were entirely lacking of oxygen consumption on basal and FCCP stimulated level. The level of ROS production was statistically higher in ddc treated cells in comparison to control as measured with DCF (2′,7′-dichlorofluorescein diacetate) and mitoSOX. The inner mitochondrial membrane potential was lower in ddc treated cells than in control cells. The TNF-α stimulated ICAM-1 synthesis, as a marker of inflammation, was lower in ddc treated EA.hy 926 cells on protein and mRNA levels. We propose that mitochondria in endothelial cells are potent target in modulation of inflammatory processes. This study was supported by the European Union with resources from the European Regional Development Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

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S7.P21

HIF-independent inhibition of mitochondrial function by DMOG: Immediate–early effects and long-term consequences

Alexander Zhdanov, Fergus Collins, Irina Okkelman, Dmitri Papkovsky
University College Cork, Ireland
E-mail address: a.zhdanov@ucc.ie

Hypoxia inducible factors (HIF-1/2) signalling brings together mitochondrial, cancer and hypoxia research. Hypoxia is often associated with cancer, however even at non-restricted O_2 supply certain mitochondrial abnormalities trigger HIF pathways and metabolic rearrangements for cancer development [1]. However, alterations in HIF signalling only partly explain the dramatic changes in mitochondrial function and cancer development. Using dimethyloxaloylglycine (DMOG), a cell-permeable compound structurally similar to α-ketoglutarate and fumarate and potent activator of HIF, we found that early inhibitory effect of this drug on the mitochondria is HIF-independent. Pre-treatment of PC12 and HCT116 cells with 0.5 mM DMOG for 0.5 h noticeably reduced mitochondrial respiration and activated glycolytic activity, measured using phosphorescent O_2 and pH sensors [2]. At this time–point the translation of HIF target genes was not activated and could not contribute to the inhibition of mitochondria. Moreover, the effect of DMOG was not abolished in the presence of a HIF inhibitor FM19G11. Exploring this finding further, we observed pronounced remodelling of the mitochondrial network, composition, size and proton gradients upon continuous exposure of cells to DMOG. Such changes as an increase in mitochondrial size, elevation of VDAC1 and decrease in COXIV levels were not sensitive to FM19G11 treatment and differed from that observed upon continuous hypoxia. Overall, our results provide a new insight in the understanding of the mechanisms underlying mitochondrial dysfunction.

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S7.P22

Possible improvement of neuronal mitochondrial bioenergetics by nobiletin involves activation of matrix substrate-level phosphorylation

Elene Zhuravliova[a], Natia Jojua[a], Nino Sharikadze[a], Elnari Zaalishvili[b], David G. Mikeladze[a]
[a]*Ilia State University, Georgia*
[b]*I. Beritashvili Center of Experimental Biomedicine, Georgia*
E-mail address: elene_zhuravliova@iliauni.edu.ge

Adult-onset deficiency of thyroid hormones causes a wide range of nervous system dysfunctions, including impairments in learning and memory. Nobiletin is a one of interesting flavonoids that has neuroprotective and neurotrophic properties and affects the cognitive function of brain. ATP synthesis, succinate dehydrogenase (SDH) and mitochondrial hexokinase (mt-HK) activities in synaptic (SM) and cell mitochondria (CM) of hippocampus of adult rats were compared among euthyroid, hypothyroid, thyroxin (T4)-treated and nobiletin-supplemented hypothyroid states. Decreased synthesis of ATP in hypothyroid rat hippocampus was observed in CM and SM, which was reversed by hormone replacement, as well as by nobiletin administration. Oligomycin-insensitive production of ATP, as well as α-ketoglutarate-dependent production of ATP was also decreased in CM and SM of hypothyroid animals and this reduction was not recovered after treatment of rats by thyroxin in SM. Only the supplementation of nobiletin increases oligomycin-insensitive and α-ketoglutarate-dependent production of ATP in both types of mitochondria. SDH activity of hypothyroid rats was lower in SM than in control rats, whereas SDH activity in CM of hypothyroid rats exceeds the control level. The activity of SDH in CM was normalized after hormone replacement or nobiletin administration, whereas in SM, only treatment of animals by T4 leads to the elevation of SDH activity. Besides, we have found that in hypothyroid rats the mt-HK

activity, which was determined with exogenic ATP, is higher than in control animals, whereas the enzyme's activity, which used endogenic ATP is lower than in CM of control animals. In both cases, the HK activity was normalized to the control levels after feeding of rats with nobiletin. These data suggest that nobiletin could enhance mitochondrial ATP synthesis in hypothyroidism through activation of matrix substrate-level phosphorylation, which may be relevant for the prevention and treatment of hypometabolic disorders.

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S7.P23

Protective effect of NIM811 – a cyclophilin inhibitor without immunosuppressive activity – in models of collagen VI muscular dystrophy

Alessandra Zulian[a,b], Erika Rizzo[a,b], Marco Schiavone[c], Elena Palma[a], Francesca Tagliavini[d], Bert Blaauw[a], Luciano Merlini[d], Nadir Mario Maraldi[d], Patrizia Sabatelli[d,e], Paola Braghetta[f], Paolo Bonaldo[g], Francesco Argenton[b], Paolo Bernardi[h]
[a]*Department of Biomedical Sciences, University of Padova, I-35131, Italy*
[b]*Consiglio Nazionale delle Rice, Italy*
[c]*Department of Biology, University of Padova, I-35131, Italy*
[d]*Laboratory of Musculoskeletal Cell Biology, Istituto Ortopedico Rizzoli, I-40136, Italy*
[e]*Consiglio Nazionale delle Rice, Italy*
[f]*Department of Molecular Medicine, University of Padova, I-35131, Italy*
[g]*Consiglio Nazionale delle Ricerche Neuroscience Institute, I-35131, Italy*
[h]*Department of Biomedical Sciences, University of Padova, Italy*
E-mail address: zulian.alessandra@gmail.com

Ullrich Congenital Muscular Dystrophy (UCMD) and Bethlem Myopathy (BM) are inherited muscle diseases due to mutations in the genes encoding the extracellular matrix protein collagen (Col) VI. Opening of the cyclosporin A-sensitive mitochondrial permeability transition pore is a causative event in disease pathogenesis, and a potential target for therapy. Here we have tested the effect of N-methyl-4-isoleucine-cyclosporin (NIM811), a non-immunosuppressive cyclophilin inhibitor, in a zebrafish model of ColVI myopathy obtained by deletion of the N-terminal region of the ColVI α1 triple helical domain, a common mutation of UCMD. Treatment with antisense morpholino sequences targeting col6a1 exon 9 at the 1–4 cell stage (within 1 hour post fertilization, hpf) caused severe ultrastructural and motor abnormalities as assessed by electron and fluorescence microscopy, birefringence, spontaneous coiling events and touch-evoked responses measured at 24–48 hpf. Structural and functional abnormalities were largely prevented when NIM811 – which proved significantly more effective and less toxic than cyclosporin A – was administered at 21 hpf. Beneficial effects of NIM811 were also detected (i) in primary muscle-derived cell cultures from UCMD and BM patients, where the typical mitochondrial alterations and depolarizing response to rotenone and oligomycin were significantly reduced; and (ii) in the Col6a1 −/− myopathic mouse model, where apoptosis was prevented and muscle strength was increased. Since the permeability transition pore of zebrafish shares its key regulatory features with the mammalian pore our results suggest that early treatment with NIM811 should be tested as a potential therapy for UCMD and BM.

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